

SEQ ID NO:1:

GGCATATTAGCTTGGGTTACTGTGAATTTTCTGACAGGTCAGCTGCATGGCCACAGA  
 CAGGAGACTGTGGGGACCTTGGACCTAGGGGGAGCCTCCACCCAAATCACGTTTCT  
 GCCCCAGTTTGAGAAAACCTCTGGAACAACTCCTAGGGGCTACCTCACTTCCTTTGA  
 GATGTTTAAACAGCACTTATAAGCTCTATACACATAGTTACCTGGGATTTGGATTGAA  
 AGCTGCAAGACTAGCAACCCTGGGAGCCCTGGAGACAGAAGGGACTGATGGGGACA  
 CTTTCCGGAGTGCCTGT

SEQ ID NO:2:

GCGGGCTGCCGCGCAAGGGTGGCGCGCGCGGTTTTCTTGTTCCTGGTCAACAAG  
 AAATGTGGAGTGTCTTGGCTGAATCCTCATACAGACAAGATCATTATGGTGCTGTTA  
 GGTGAAAAAGTGATATAATAAGGAACCAAGGAGAAAATTCAGAAGGAAAGAAA  
 AAATTGCCTCTGCAGGTGTGCGAGCAGGATTGCTTCTGCAACAAAAGCCTCCACCCA  
 GCCACATCTTGGGAAAAGAATGGCCACTTCTTGGGGCACAGTCTTTTTCATGCTGGT  
 GGTATCCTGTGTTTGCAGCGCTGTCTCCACAGGAACCAGCAGACTTGGTTTGAGGG  
 TATCTTCTGTCTTCCATGTGCCCCATCAATGTCAGCGCCAGCACCTTGTATGGAATT  
 ATGTTTGATGCAGGGAGCACTGGAACCTCGAATTCATGTTTACACCTTTGTGCAGAAA  
 ATGCCAGGACAGCTTCCAATTCTAGAAGGGGAAGTTTTTGATTCTGTGAAGCCAGGA  
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 AAAGGCAACAGCAGGACTACGTTACTGCCAGAACACAAAGCCAAGGCTCTGCTCT  
 TTGAGGTAAAGGAGATCTTCAGGAAGTCACTTTTCTGGTACCAAAGGGCAGTGTTA  
 GCATCATGGATGGATCCGACGAAGGCATATTAGCTTGGGTTACTGTGAATTTTCTGA  
 CAGGTCAGCTGCATGGCCACAGACAGGAGACTGTGGGGACCTTGGACCTAGGGGGA  
 GCCTCCACCCAAATCACGTTTCTGCCCCAGTTTGAGAAAACCTCTGGAACAACTCCT  
 AGGGGCTACCTCACTTCTTTGAGATGTTTAAACAGCACTTATAAGCTCTATACACAT  
 AGTTACCTGGGATTTGGATTGAAAGCTGCAAGACTAGCAACCCTGGGAGCCCTGGA  
 GACAGAAGGGACTGATGGGCACACTTTCCGGAGTGCCTGTTTACCGAGATGGTTGG  
 AAGCAGAGTGGATCTTTGGGGGTGTGAAATACCAGTATGGTGGCAACCAAGAAGGG  
 GAGGTGGGCTTTGAGCCCTGCTATGCCGAAGTGCTGAGGGTGGTACGAGGAAAAC  
 TCACCAGCCAGAGGAGGTCCAGAGAGGTTCTTCTATGCTTTCTCTTACTATTATGA  
 CCGAGCTGTTGACACAGACATGATTGATTATGAAAAGGGGGGTATTTTAAAAGTTGA  
 AGATTTTGAAAGAAAAGCCAGGGAAGTGTGTGATAACTTGGAACCTTACCTCAG  
 GCAGTCCTTTCTGTGCATGGATCTCAGCTACATCACAGCCCTGTTAAAGGATGGCT  
 TTGGCTTTGCAGACAGCACAGTCTTACAGCTCACAAAGAAAGTGAACAACATAGAG  
 ACGGGCTGGGCTTGGGGGCCACCTTTACCTGTTGCAGTCTCTGGGCATCTCCCAT  
 TGAGGCCACGTACTTCTTGGAGACCTGCATTTGCCAACACCTTTTTAAGGGGAGGA  
 GAGAGCACTTAGTTTCTGAACTAGTCTGGGGACATCCTGGACTTGAGCCTAGAGATT  
 WRGTTAATTAASCGGCCGAGCTTATCCTTWATRAGGTAATTTACTTGCMTGGCCGCG  
 TTTACACGTCGTGATGGNAAACCTGCGTCCCAACTAACGCTTGASAMATCCCCTTCG  
 CAGCTGCGATACCAAAGCCGACGACGCCTTCCACAGTGCCA

Figure 1

SEQ ID NO:3:

MATSWGTVFFMLVVSCVCSAVSHRNQQTWFEGIFLSSMCPINVSASTLYGIMFDAGSTG  
TRIHVYTFVQKMPGQLPILEGEVFDVSKPGLSAFVDQPKQGAETVQGLLEVAKDSIPRSH  
WKKTPVVLKATAGLRLLPEHKAKALLFEVKEIFRKSPFLVPKGSVSIMDGSDEGILAWV  
TVNFLTGQLHGHRQETVGTLDLGGASTQITFLPQFEKTLQTPRGYLTSEMFNSTYKLY  
THSYLGFGGLKAARLATLGALETEGTDGHTFRSACLPRWLEAEWIFGGVKYQYGGNQEG  
EVGFEP CYAEVLRVVRGKLHQPEEVQRGSFYAFSYYYDRAVDTDMIDYEKGGILKVED  
FERKAREVCDNLENFTSGSPFLCMDLSYITALLKDGFGFADSTVLQLTKKVNNIETGWA  
LGATFHLLQSLGISH

Figure 2

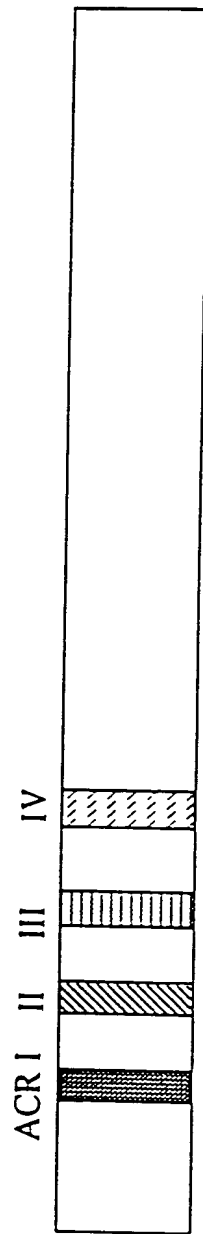
CD39Human.seq	M E D T K E S N V K T F E S S N I C A T L G F S S I T A V I A L L A - - - - -	38
246 prot	M A T L S W G L V F - - - - - F M L V V S C V C S A M S H R N C Q T W F E S I F	34
CD39Human.seq	Q N K - - - - - A L P E N V K Y G I V L D A G S S K T S L Y I Y K P A E E N D	74
246 prot	L S S M O P I N V S A S T L Y G D M F L A G S T Q T R I H V Y T F V Q K M P Q Q	74
CD39Human.seq	T G V V H Q V E E C R V K Q P G I S K F V Q K V N E I G I V L T D C M E P A R E	114
246 prot	L P L L E G E V F D S I V K - P G L S A E V D Q P K Q G A E T V C C L L E V A R D	112
CD39Human.seq	V I P R S Q H Q E T P V Y L G A T A G M R L L E M E S E E L A D R K L D V E R	154
246 prot	S I P R S H W K K T P V I V L K I A T A G L R L L - - - P L E H K I A K A L L F E M K E	150
CD39Human.seq	S L S N Y P F D F Q - - - G A R I I T G O E E G A Y G W I T I N Y L L O K F S Q K	192
246 prot	I F R K S P E L V P K G S V S L M D G S D E G I L A W V T V N F L T Q Q L - - -	187
CD39Human.seq	T R W F S L V P Y E T N N Q E T E G A L D L G G A S T Q V T F V P Q - - - - -	231
246 prot	- - - - - H G H R Q E T V G T L D L G G A S T Q T T E L P Q F E K T L E	216
CD39Human.seq	S P D N A - - - - - L O F R L Y G K D Y N V Y T H S F L C Y G K D O A L W C K L A K D	269
246 prot	Q T P R G Y L T S E E M F N S T Y K L Y T H S Y L G F G L K A A - - - R L A T L	255
CD39Human.seq	T O V A S N E I L R D F C F H P G Y K K V V N V S D L Y K T P C T K R - - - F E M T	303
246 prot	G A L E T E G - - - - - - - - - - - - - - - T D G H T F R S A C L P W L E A E	280
CD39Human.seq	L P F O O F - - - - - E I Q G I G N Y O O C H O S I L E L F N T S Y C P Y S Q	342
246 prot	W I E G G V K Y Q Y G G N Q E I G E V G F E P C Y A E V L R V V R G K - - - - -	314
CD39Human.seq	C A F N G I F L P P L O G E F G A F S A F - - - Y F V M K F L N L T S E K V S Q E	360
246 prot	- - - - - L H Q P E E V Q R I G S E Y A E S Y V Y D R - - - A V D T D M I D Y E	345
CD39Human.seq	K V T E M - - - - - M K K F C A Q P W E - - - E I K T S Y A G V K E K Y L S E Y C F S G T	417
246 prot	K G G I L K V E D E F E R K A R E V C D N L E N F T S G S P - F L - - - C M D L S	381
CD39Human.seq	Y I L S L L L O G Y H F T A D S W E H I H F I G K I O G S D A G W T L G Y M L N	457
246 prot	Y I T A L L K D G F G F A D S T - - - V L Q L T K K V N N I E T G W A L G A T F H	419
CD39Human.seq	L T N M I P A E Q P L S T P L S H S T Y V F L M V L F S L V L F T V A I I G L L	497
246 prot	L L Q S L G I S H	428
CD39Human.seq	I F H K P S Y F W K D M V	510
246 prot		428

Figure 3

1	M A T S W G T V F F M L V V S C V E S A V E H R N Q Q T W F E C I F L S S M C P	246	prot
1	M A T S W G A V F - M L L I A Q V G S T V F Y I R E Q Q T W F E C I F L S S M C P		mur ncpase
41	I N V S A L S T L Y G I M F D A G S T G T R I H V Y T F V Q A M R C Q C L P F L E C	246	prot
40	I N V S A G T F I Y G I M F D A G S T G T R I H V Y T F V Q K T A G C L P F L E C		mur ncpase
81	E V F D S V K P G L S A F V D Q P K Q G A E T V Q Q L L E V A K D S I P R S E W	246	prot
80	E I F D S V K P G L S A F V D Q P K Q G A E T V Q I E L L E V A K D S I P R S E W		mur ncpase
121	K K T P V V L K A T A G L R L L P E H K A K A L E E F E V K E I F R K S P F L V P	246	prot
120	E R I T P V V L K A T A G L R L L P E Q K A Q A L L E V E E I F R K S P F L V P		mur ncpase
161	K G S V S I M D G S D E G I L A W V T V N F L T G Q L H G E R Q E T V G T L D L	246	prot
50	D G S V S I M D G S Y I E G I L A W V T V N F L T G Q L H G R I G Q E T V G T L D L		mur ncpase
201	G G A S T Q I T F L P Q F E K T L E Q T P R G Y L T S F E M F N S T Y K L Y T H	246	prot
200	G G A S T Q I T F L P Q F E K T L E Q T P R G Y L T S F E M F N S T F K L Y T H		mur ncpase
241	S Y L G F G L K A A R L A T L G A L E T E G T D G H T F R S A C L P R W L E A E	246	prot
240	S Y L G F G L K A A R L A T L G A L E A K G T D G H T F R S A C L P R W L E A E		mur ncpase
281	W I F G G V K Y Q Y G G N Q E G E V G F E P C Y A E V L R V V R G K L H Q P E E	246	prot
280	W I F G G V K Y Q Y G G N Q E G E M G F E P C Y A E V L R V V G K L H Q P E E		mur ncpase
321	V Q R G S F Y A F S Y Y Y D R A V I D T D M I D Y E K G G I L K V E D F E R K A R	246	prot
320	V R G S A F Y A F S Y Y Y D R A A D T H I L I D Y E K G G V L K V E D F E R K A R		mur ncpase
361	E V C D N L E I N F T S G S P F L C M D L S Y I T A L L K D G E G F A D S T V L I Q	246	prot
360	E V C D N L G S F S S G S P F L C M D L T Y I T A L L K D G L G F A E R R P L T		mur ncpase
401	L T K K V N N I E T G W - A L G A T F - - - - - H L L O S L G I S H	246	prot
400	- A H K E S E Q H R D W L G L G G H L S P A P V S G H H Q L R P S S T S E A C I		mur ncpase
426		246	prot
439	S E P V F S Q E G V D S E T F S D L S G K A W P E T R		mur ncpase

Figure 4

# Apyrase Conserved Regions in CD39-L4



ACR I	CD39-L4	F	DAGST	G	T	R	I
	CD39-L4 mutant	L	DAGST	H	T	S	I
	CD39	L	DAGSS	H	T	S	L
ACR II	CD39-L4	PV	V	L	K	ATAGLRLL	
	CD39-L4 mut	PV	Y	L	G	ATAGLRLL	
	CD39	PV	Y	L	G	ATAGMRLL	
ACR III	CD39-L4	M <sub>1</sub>	D	S	DEGI	L	AWVTVN
	CD39-L4 mut	M <sub>1</sub>	T	Q	DEGI	F	AWVTVN
	CD39	L	L	Q	EEGA	Y	GWITIN

FIG. 5

Nucleotide sequence of the CD39-L4 mutant ACRIII (SEQ ID NO:6). The nucleotide changes have been highlighted. The G to A and A to C changes at positions 502 and 503 produce a Thr, the T to C, C to A and C to A changes at positions 508-510 result in a Gln and the A to C changed at position 525 result in a Phe.

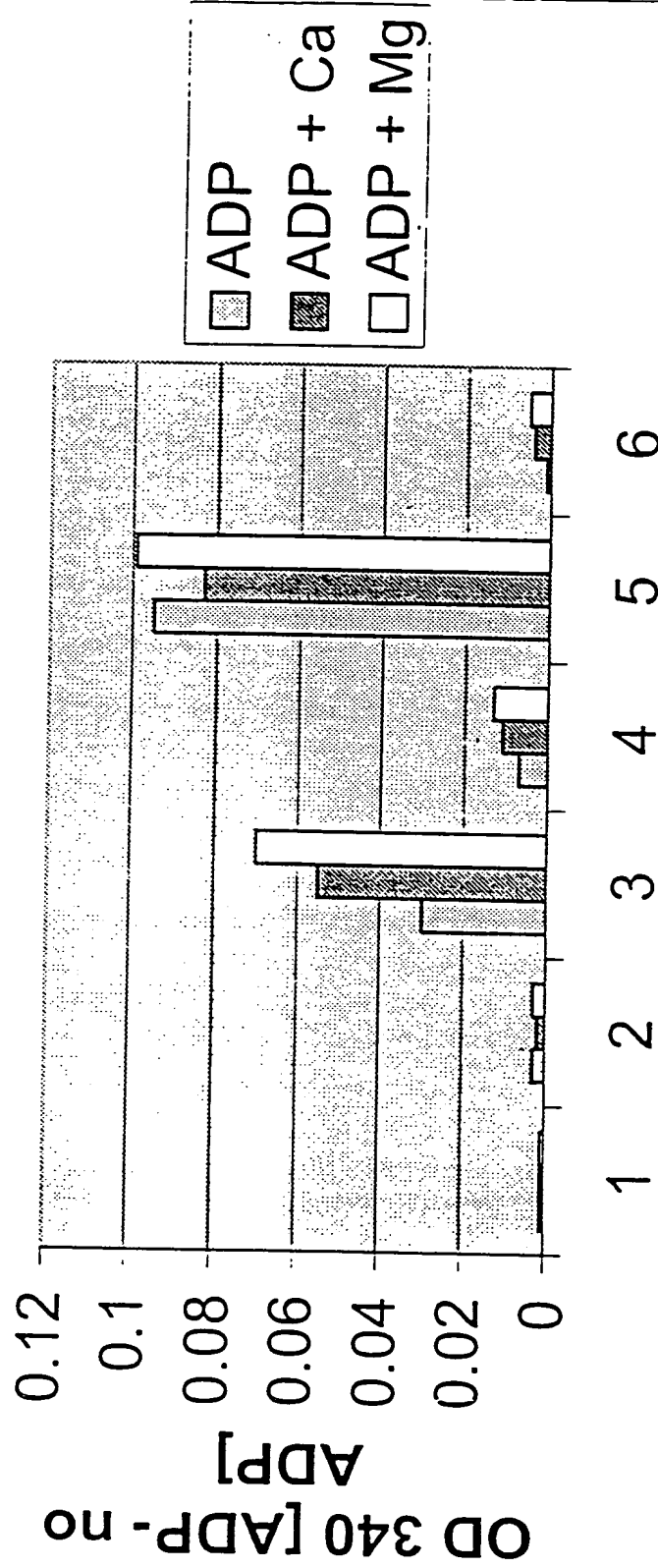
ATGGCCACTTCTTGGGGCACAGTCTTTTTTCATGCTGGTGGTATCCTGTGTTTGCAGCGCTGTCT  
 CCCACAGGAACCAGCAGACTTGGTTTGAGGGTATCTTCCTGTCTTCCATGTGCCCCATCAATGT  
 CAGCGCCAGCACCTTGTATGGAATTATGTTTGTATGCAGGGAGCACTGGAACCTCGAATTCATGTT  
 TACACCTTTGTGCAGAAAATGCCAGGACAGCTTCCAATTCTAGAAGGGGAAGTTTTTGATTCTG  
 TGAAGCCAGGACTTTCTGCTTTTGTAGATCAACCTAAGCAGGGTGCTGAGACCGTTCAAGGGCT  
 CTTAGAGGTGGCCAAAGACTCAATCCCCCGAAGTCACTGGAAAAAGACCCCAAGTGGTCTTAAAG  
 GCAACAGCAGGACTACGCTTACTGCCAGAACACAAAGCCAAGGCTCTGCTCTTTGAGGTAAAGG  
 AGATCTTCAGGAAGTCACCTTTCCTGGTACCAAAGGGCAGTGTTAGCATCATGACTGGACAAGA  
 CGAAGGCATATTGCTTGGGTTACTGTGAATTTTCTGACAGGTCAAGTGCATGGCCACAGACAG  
 GAGACTGTGGGGACCTTGGACCTAGGGGGAGCCTCCACCCAAATCACGTTCTGCCCCAGTTTG  
 AGAAAACCTCTGGAACAACTCCTAGGGGCTACCTCACTTCTTTGAGATGTTTAAACAGCACTTA  
 TAAGCTCTATACACATAGTTACCTGGGATTGGATTGAAAGCTGCAAGACTAGCAACCCTGGGA  
 GCCCTGGAGACAGAAGGGACTGATGGGCACACTTTCGGGAGTGCCTGTTTACCGAGATGGTTGG  
 AAGCAGAGTGGATCTTTGGGGGTGTGAAATACCAGTATGGTGGCAACCAAGAAGGGGAGGTGGG  
 CTTTGAGCCCTGCTATGCCGAAGTGCTGAGGGTGGTACGAGGAAAACCTTCACCAGCCAGAGGAG  
 GTCCAGAGAGGTTCTTCTATGCTTTCTCTTACTATTATGACCGAGCTGTTGACACAGACATGA  
 TTGATTATGAAAAGGGGGGTATTTTAAAGTTGAAGATTTTGAAGAAAAGCCAGGGGAAGTGTG  
 TGATAACTTGGAACCTTCACC TCAGGCAGTCCTTTCCTGTGTCATGGATCTCAGCTACATCAC  
 AGCCCTGTTA AAGGATGGCTTTGGCTTTGCAGACAGCACAGTCTTACAGCTCACAAAGAAAGT  
 GAAC AACATAG AGACGGGCTGGGCCTTGGGGGCCACCTTTCACCTGTTGCAGTCTCTGGGCA  
 TCTCCATTGA

Amino acid sequence of CD39-L4 mutant ACR III (SEQ ID NO:7). The amino acid changes are D to T (a.a. 168), S to Q (a.a. 170) and L to F (a.a. 175). The changes are shown in bold.

MATSYGTVFFMLVVSCVCSAVSHRNQQTWFEGIFLSSMCPINVSASTLYGIMFDAGSTGT  
 RIHVYTFVQKMPGQLPILEGEVFDSEVKPGLSAFVDQPKQGAETVQGLLEVAKDSIPRSHW  
 KKTPVVLKATAGLRLLPEHKAKALLFEVKEIFRKSPFLVPKGSVSIMTGQDEGIFAWVTV  
 NFLTGQLHGHQRQETVGTLDLGGASTQITFLPQFEKTLQTPRGYLTSEFMFNSTYKLYTH  
 SYLGFLKAARLATLGALETEGTDGHTFRSACLPRWLEAEWIFGGVKYQYGGNQEGEVGF  
 EPCYAEVLRVVRGKLHQPEEVQRGSFYAFSYYYDRAVD TDMIDYEKGKILKVEDFERKAR  
 EVCDNLENFTSGSPFLCMDLSYITALLKDGFGFADSTVLQLTKKVNNIETGWALGATFHL  
 LQSLGISH

FIG. 6

**FIG. 2 ADPase activity of CD39-L4  
ACR mutants**



1) ACR I; 2) ACR II; 3) ACR III; 4)  
Wild Type; 5) CD39; 6) Vector

**FIG. 7**

SEQ ID No-3	MATSWG	-----VFFMLVVSVCVCSAVSHRNQQTWFEGIFLSSMCPINVSAS	46	
SEQ ID No-5	MATSWG	-----VFFMLVVSVCVCSAVSHRNQQTWFEGIFLSSMCPINVSAS	46	
CD39Human.seq	MEDTKESNVKTFCSKNILAILGFS	SIIVIA-----LLAVGLTQ--NKALPEN	46	
SEQ ID No-3	TLYGIMFDAGSTGTRI	HVYTFVQKMPGQLPILEGEVFDVSK	100	
SEQ ID No-5	TLYGIMFDAGSTGTRI	HVYTFVQKMPGQLPILEGEVFDVSK	100	
CD39Human.seq	VKYGIVLDAGSSHT	SLYIKWPAEKENDTGVVHQVEECRVKGP	101	
SEQ ID No-3	AETVQGLLEVAKDSIPRSHWKKTPV	VVLKATAGLRLL	152	
SEQ ID No-5	AETVQGLLEVAKDSIPRSHWKKTPV	VVLKATAGLRLL	152	
CD39Human.seq	GIYLTDCMERAREV	IPRSQHQTPTVYLGATAGMRLLRMESEELADRVL	156	
SEQ ID No-3	RKSPFLVPKGSVSI	MDGSDGILAWVTVNFLTGGQL	195	
SEQ ID No-5	RKSPFLVPKGSVSI	MDGSDGILAWVTVNFLTGGQL	195	
CD39Human.seq	SNYPFDQ--GAR	ITIGQEEGAYGWITINYLGLGKFSQKTRWFSIVPYETNNQET	209	
SEQ ID No-3	GTLDLGGASTQIT	FFLPQFEKTL	EQTPRGYLTSEFEMFNSTYKLYTHSYLGGFLKAA	250
SEQ ID No-5	GTLDLGGASTQIT	FFLPQFEKTL	EQTPRGYLTSEFEMFNSTYKLYTHSYLGGFLKAA	250
CD39Human.seq	GALDLGGASTQV	TFVPO--NQTI	ESPDNA--LQERLYGKDYNVYTHSYFLCYGKDQAN	261
SEQ ID No-3	-----RLATLGALETEG	-----	TDGHTFRSACLPRLWEAEWIFGGVK	287
SEQ ID No-5	-----RLATLGALETEG	-----	TDGHTFRSACLPRLWEAEWIFGGVK	287
CD39Human.seq	LWQKLAKD	IQVASNEILRDPCHFPGYKKVNVSDLYKTPCTKR--FEMTLPEQQF--	314	
SEQ ID No-3	YQYGGNQEGEVGF	EPCYAEVLRVVRGK	-----LHQPEEVQSGSFYAFS	330
SEQ ID No-5	YQYGGNQEGEVGF	EPCYAEVLRVVRGK	-----LHQPEEVQSGSFYAFS	330
CD39Human.seq	-----EIQG	IGNYQQCHQSIL	ELFNSTSYCPYSQCAFNGLFLPLQGGDFGAFSAF--	363
SEQ ID No-3	YYYDR	-----AVDTMDIDYEKGGILKVEDFERKAREVCDNLENFTSGSP--FL--	CM	378
SEQ ID No-5	YYYDR	-----AVDTMDIDYEKGGILKVEDFERKAREVCDNLENFTSGSP--FL--	CM	378
CD39Human.seq	-YFV	MKFLNLTSEKVSQEKVTEM--MKKFC	CAQPWLE--EIKTSYAGVKEKYLSEYCF	414
SEQ ID No-3	DLSYITALLKDGFGFADST	-----	VLQLTKKVNNIETGW	412
SEQ ID No-5	DLSYITALLKDGFGFADST	-----	VLQLTKKVNNIETGW	412
CD39Human.seq	SGTYILSLL	LQGYHFTADSWEHIFIGKIQGSDAGWTLGYMLNLT	-----	402
SEQ ID No-3	ALGATFHLLQSLGISH	-----	-----	459
SEQ ID No-5	-----VLR	-----	-----	429
CD39Human.seq	-----NMIPAEQPLSTPLSHSTYVFLMVLFSLVLTVA	IIGLLIFHKP	-----	405

FIG 8



Fig. 9

Fig. 9